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**Mitochondria and the hallmarks of cancer**

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## Abstract

Mitochondria have been traditionally viewed as the powerhouse of the cell due to their major role in the generation of ATP. More recently, mitochondria have also been demonstrated to have key roles in a variety of other processes such as apoptotic cell death and inflammation. Here we review the different ways in which mitochondrial functions impact on cancer. While cancer is comprised of diverse types, distinct hallmarks have been defined that are applicable to most cancer types. We provide an overview of how mitochondria impact on specific hallmarks; these include evasion of cell death, deregulated bioenergetics, genome instability, tumour promoting inflammation and metastasis. In addition to discussing the underlying mitochondrial roles in each of these processes, we also highlight the considerable promise of targeting mitochondrial functions in order to improve cancer treatment.

Introduction

Cancer is a highly diverse disease that is comprised of over two hundred different types. Nevertheless, despite this diversity, cancer displays stereotypical traits. These hallmarks of cancer, as defined by Hanahan and Weinberg, include, but are not limited to, resistance to cell death, tumour-promoting inflammation and deregulated metabolism [1]. It is increasingly apparent that mitochondria play key roles in most, if not all of the hallmarks of cancer. By virtue of their key biosynthetic functions, notably in the generation of ATP, mitochondria are essential for life. More recently, mitochondria have been implicated as central regulators of other processes including cell death, inflammation, immunity and migration. Perhaps unsurprisingly, these functions often interconnect with the metabolic roles of mitochondria. In this review we will discuss the roles that mitochondria play in various hallmarks of cancer. For reasons of brevity we will focus on mitochondrial regulation of cell death, deregulated metabolism, inflammation, genome-instability and migration. Our discussion, will serve to highlight the roles mitochondria play in cancer development and progression as well as describing ways in which these can be exploited for therapeutic benefit.

Mitochondria and evasion of cell death

Cell death acts as a potent tumour suppressor mechanism [2]. The best-described form of programmed cell death is apoptosis; this process requires caspase protease activity leading to rapid cell death this is associated with characteristic morphological and biochemical changes. Key tumour suppressor proteins such as p53 engage

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3 apoptosis in damaged cells, for example following DNA-damage, to prevent cells from  
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5 becoming transformed. Apoptosis also prevents tumour progression in other ways,  
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7 such as killing cells in nutrient-poor environment or killing cancer cells that detach  
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9 from extracellular matrix and metastasize, a form of death called anoikis. Following  
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11 most apoptotic stimuli, mitochondria are essential for the execution of cell death.  
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13 Through a process called mitochondrial outer membrane permeabilisation or MOMP,  
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15 soluble proteins are released from the mitochondrial intermembrane space that  
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17 actively kill the cell [3] (Figure 1). Chief amongst the killer proteins released from the  
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19 mitochondria is the electron transport protein cytochrome c; following mitochondrial  
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21 release cytochrome c binds the adaptor molecule APAF-1 triggering caspase  
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23 activation and rapid cell death. Importantly, MOMP often leads to cell death  
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25 irrespective of caspase activity, and, as such, represents a point-of-no-return [4].  
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34 Because of its deadly consequences, mitochondrial outer membrane integrity is  
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36 highly regulated, mainly through protein-protein interactions between pro- and anti-  
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38 apoptotic members of the Bcl-2 protein family [5]. Following an apoptotic stress, pro-  
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40 apoptotic BH3-only members of the Bcl-2 protein family relay the stress signal to the  
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42 mitochondria. Here they activate two key activator Bcl-2 proteins, called BAX and  
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44 BAK, that, through a poorly understood mechanism, permeabilise the mitochondrial  
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46 outer membrane, triggering cell death. Anti-apoptotic BCL-2 proteins inhibit  
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48 apoptosis by binding and sequestering BH3-only proteins or by binding and inhibiting  
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50 activated BAX and BAK (Figure 1).  
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Cancer cells evade apoptosis in numerous ways [5]. One common way is by failing to respond to an apoptotic stimulus, for example loss of p53 can make cells more resistant to DNA-damage induced apoptosis. Alternatively, cancer cells can up-regulate anti-apoptotic proteins, for example up-regulation of anti-apoptotic Bcl-2 proteins is commonly observed across diverse tumour types. Importantly, beyond promoting cancer, inhibition of mitochondrial apoptosis can also cause resistance to anti-cancer treatments, many of which kill cancer cells through the induction of cell death [6]. Nevertheless, although inhibition of cell death may promote cancer, many cancer cells (particularly haematological malignancies) display increased apoptotic sensitivity relative to their healthy, cellular counterparts [7]. Cells in this state, are termed "primed-to-die" and are dependent on anti-apoptotic Bcl-2 function for survival. Importantly, primed cancer cells are sensitised (relative to normal tissue) to apoptosis inducing therapies [8]. This is exploited by new targeted therapies called BH3-mimetics that, analogous to BH3-only proteins, neutralise anti-apoptotic BCL-2 function [9, 10]. In doing so, BH3-mimetics either trigger or sensitise cells to death.

**Mitochondria and deregulated cellular energetics**

Mitochondria are best known for their essential role in powering the cell, generating energy in the form of ATP through oxidative phosphorylation (OXPHOS). Additionally, mitochondria are key to various other biosynthetic processes, such fatty acid synthesis. Over recent years, intense interest has focused on cancer metabolism since far from simply having higher rates of metabolism, which might be expected due to increased proliferative rates, cancer cells often display alterations in metabolic pathways [11]. These alterations can both drive tumourigenesis, by generating so-

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3 called oncometabolites, or are required to facilitate tumour cell survival in hypoxic or  
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5 nutrient poor environments. Importantly, metabolic re-wiring of tumour cells offers  
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7 cancer-specific drug targets. Here we provide an overview of some of the ways in  
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9 which mitochondrial metabolic pathways are deregulated in cancer.  
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15 In mitochondrial respiration, the carbon fuel acetyl-CoA, generated via  
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17 glycolysis or fatty acid oxidation, powers the tricarboxylic acid cycle within the  
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19 mitochondrial matrix. The TCA generates NADH and FADH<sub>2</sub> that transfer electrons to  
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21 the mitochondrial respiratory chain. This, in turn, leads to the generation of an  
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23 electrochemical gradient over the mitochondrial innermembrane, the energy of  
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25 which generates ATP production by ATP synthase. Besides mitochondrial respiration,  
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27 cells can produce ATP through the process of glycolysis. In terms of ATP generation  
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29 glycolysis is much less efficient than mitochondrial respiration, producing 2 and 36  
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31 molecules of ATP per glucose molecule respectively. Nevertheless, glycolysis serves to  
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33 sustain ATP generation (and cell viability) under conditions where mitochondrial  
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35 respiration is impaired - for example, under low oxygen conditions. The best  
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37 described, and almost universal metabolic adaptation of cancer cells is the  
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39 predominant use of glycolysis to generate ATP even under aerobic conditions - called  
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41 the Warburg effect [11]. Exactly why cancer cells rely on glycolysis in this way remains  
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43 unclear, though a likely reason is the ability of glycolysis, via intermediary pathways,  
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45 to generate nucleotides, lipids and amino acids, all of which are required for rapid  
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47 proliferation. In addition to the Warburg effect, as we will discuss now, more specific  
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49 alterations in metabolic pathways have been identified in certain cancers, many of  
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which track back to loss or gain of function mutations in key enzymes involved in mitochondrial metabolism.

*Mutant metabolic enzymes as oncogenic drivers*

Isocitrate Dehydrogenase (IDH) is an enzyme that catalyses the oxidation of isocitrate to  $\alpha$ -ketoglutarate, a reaction occurs in the mitochondria (as part of the TCA) as well as in the cytoplasm. Recurrent mutations in two isoforms of IDH, IDH1 and IDH2 have been identified in a variety of tumours, most commonly in acute myeloid leukemia (AML) and glioblastoma [12-14]. These mutations in IDH, impart a gain of function effect, where  $\alpha$ -ketoglutarate is further metabolised to 2-hydroxyglutarate (2-HG). 2-HG represents an oncometabolite that competitively inhibits enzymatic reactions that require  $\alpha$ -ketoglutarate as a co-substrate (enzymes called 2OG dioxygenases) [13-15]. This has pleiotropic effects all of which possess potential oncogenic impact, including effects on epigenetic regulation, histone methylation, collagen synthesis and hypoxia induced factor signalling [13-17]. Due to their cancer cell specificity, mutant IDH1/2 potentially represent ideal therapeutic targets. Accordingly, inhibitors that specifically target mutant IDH1 have recently been developed and are currently under clinical evaluation [12].

Fumarate hydratase (FH) is a crucial enzyme in the TCA cycle, where it converts fumarate to malate. Loss-of-function mutations to FH have been found in renal cell cancer, causing an increase in fumarate and decrease in malate and citrate [18]. Fumarate is thought to exert oncogenic effects through its ability to activate HIF signaling, by directly inhibiting prolyl 4-hydroxylase (PHD) function - the negative



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3 regulator of HIF [19]. A key question that arises is how can cells survive the disruption  
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5 of TCA cycle function by loss of fumarate hydratase (FH)? Addressing this, combined  
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7 approaches of metabolomic analyses with computational modelling, revealed that  
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9 haem oxygenase was required for survival of FH deficient cells, allowing the  
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11 breakdown of accumulated TCA metabolites and partial restoration of NADH  
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13 production [20]. Importantly, inhibiting haem oxygenase activity specifically killed Fh  
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15 deficient tumour cells leading to the prediction that therapeutic exploitation of this  
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17 synthetic lethal relationship should display tumour specific killing.  
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24 Cancer promoting mutations in mitochondrial respiratory complex II have also  
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26 been identified. Complex II, is comprised of succinate dehydrogenase (SDH) activity  
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28 that links the TCA cycle to the respiratory chain by providing electrons generated  
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30 during the conversion of succinate. Complex II consists of 4 SDH subunits, mutations  
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32 of which have been reported in paragangliomas, cancers of the neuroendocrine  
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34 system [21]. In a manner analogous to fumarate, accumulated succinate inhibits  
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36 PHD activity leading to HIF activation and pro-oncogenic effects.  
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#### 42 43 *Interplay of mitochondrial energetics and tumour suppressor pathways* 44

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46 Alterations of mitochondrial metabolism via direct mutation of key metabolic  
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48 enzymes appears relatively rare. More commonly, mitochondrial energetics are  
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50 affected by an almost bewildering array of signaling mechanisms. Over recent years,  
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52 both oncogenic and tumour suppressor pathways have been shown to directly effect  
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54 a variety of mitochondrial metabolic pathways, strongly supporting the notion that  
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56 deregulated metabolism underpins cancer development and progression. As an  
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example, here we will focus on the tumour suppressor protein, p53, the activity of which is inhibited in the vast majority of cancers [22].

p53 exerts tumour suppressor function through its role as a transcription factor, where it up-regulates many genes; these, in turn, lead to an array of tumour suppressor functions that include induction of cell-cycle arrest, senescence and apoptosis [23]. Amongst other stresses, such as genotoxic insults, nutrient stress can activate p53 through numerous means, commonly through activation of AMP-activated protein kinase (AMPK) leading to direct phosphorylation and stabilisation/activation of p53 [24]. Once activated, p53 limits glycolysis through at least two means; one is by up-regulating a protein called TIGAR that hydrolyses fructose-2,6-biphosphate - an allosteric activator of the key glycolytic enzyme PFK1 [25]. Secondly, p53 expression can down-regulate several glycolytic enzymes. Beyond reducing the amount of glycolysis, p53 also enhances the conversion of pyruvate to acetyl-CoA allowing it to enter the TCA cycle and enhance mitochondrial respiration [26]. In addition, p53 directly enhances mitochondrial respiration in different ways such by up-regulating SCO2, a protein that promotes respiratory complex formation and OXPHOS [27, 28].

Clearly, using p53 as an example, there are many ways in which a tumour suppressor can impinge on mitochondrial metabolism. In the case of p53, exactly what (if any) of these aspects is important for tumour suppression is difficult to ascertain, namely due to the additional tumour suppressive functions that p53 also possesses.

### Mitochondria and genomic integrity

Genome instability and mutations play multiple roles in tumourigenesis ranging from promoting cellular transformation through to driving acquired treatment resistance. The causes of genome instability are varied and arise from exogenous (such as UV light) as well as endogenous insults. Here we will discuss the various ways in which, mitochondrial functions can impact on genome integrity as well as discussing the potential role of mitochondrial genome stability in cancer.

### *Mitochondrial Reactive Oxygen Species*

Mitochondria represent a major source of DNA-damaging reactive oxygen species (ROS). During oxidative phosphorylation, as a consequence of incomplete reduction of oxygen, a variety of ROS are generated that can attack both proteins and nucleic acids. The nucleotide guanine is particularly susceptible to ROS-mediated oxidation, where it is converted to 8-hydroxyguanine [29]. Although our cells have an array of effective DNA-damage response mechanisms, over time accumulated ROS-mediated damage can lead to various genomic alterations including mutations, deletions and chromosomal translocations (Figure 2A). A number of studies connect mitochondrial ROS production to genome instability and its' tumour promoting effects. For example, loss of the mitochondrial anti-oxidant protein, SOD2 *in vitro* potently induces genome instability [30]. Moreover, SOD2 heterozygote mice often develop late onset mammary tumours, implicating mitochondrial ROS as an oncogenic-driver of these tumours [30]. Supporting these findings, inactivating mutations in mitochondrial proteins succinate dehydrogenase (complex II) as well as loss of the Sirt4 have also

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been linked to ROS production and genome instability [31] (Figure 2B). Whilst these studies paint a picture that ROS are deleterious, it is important to note that ROS also play a variety of key signalling functions in various physiological processes [32].

*Mitochondrial DNA Integrity and Cancer*

Although the vast majority of mitochondrial proteins are encoded by the nuclear genome, the mitochondrial genome encodes for various proteins that essential for respiratory function as well as tRNAs and ribosomal subunits that are required for mitochondrial protein synthesis. Relative to nuclear DNA, mitochondria DNA (mtDNA) may be more susceptible to DNA-damage for different reasons such as its closer physical proximity to ROS generated via OXPHOS as well as the reduced capacity of the cell to repair DNA-damage relative to nuclear DNA. In a feed-forward mechanism, potentially mutations in mtDNA leading to reduced OXPHOS efficiency and increased ROS could have a knock-on effect on both nuclear and mitochondrial genome integrity (Figure 2A). Nevertheless, while numerous somatic and germline mitochondrial mutations have been identified in diverse human cancers, it remains controversial as to whether these are due to causal or bystanders in tumour development. Directly testing this hypothesis is extremely challenging due to the lack of means to easily modify the mitochondrial genome. However, some data supports a role for maintenance of mitochondrial genome integrity in the regulation of oncogenesis. For example, cytoplasts (cells lacking nuclear DNA) that bear an mtDNA mutation in the gene encoding ND6 (complex I subunit) have been fused to cells with low tumorigenicity [33]. This resulted in high ROS induction leading to increased tumour outgrowth and apoptotic resistance. Mitochondrial transcription factor A

(TFAM) is required for replication and expression of mitochondrial DNA. While complete TFAM loss is incompatible with life, heterozygous deletion is tolerated with minimal mtDNA deletion and no overt phenotype [34]. Mitochondria from *Tfam*<sup>+/-</sup> mice are capable of producing increased ROS (relative to mitochondria from wild-type mice)[34]. Most importantly, *Tfam* heterozygosity promotes tumourigenesis in a mouse model of colorectal cancer in a manner that can be inhibited by transgenic expression of the anti-oxidant enzyme, mitochondrial-targeted catalase (mCAT) [34]. Similarly, decreased mtDNA stability and ROS production have also been linked to the genetic ablation of the nuclear encoded mitochondrial RNA helicase (SUV3). Mice that are heterozygous for SUV3 exhibit short lifespan and increased tumourigenicity [35].

While these data demonstrate that affecting mitochondrial genetic stability can promote cancer (possibly through production of ROS) whether this actually happens remains controversial. Typically, hundreds of copies of mtDNA exist per cell leading to a mixed pool of mtDNA genotypes in each cell - such a state is termed heteroplasmy. Some studies have found selection of specific mtDNA in tumour types, suggesting a causal role for mtDNA in tumour suppression. For example, an oncocytoma patient with a frameshift mutation in the complex I subunit ND5 displayed enrichment of mtDNA with this mutation in tumour compared with healthy tissue, suggesting that it conferred selective advantage during tumourigenesis [36]. Supporting this, an additional study found that the ND5 frameshift mutation fosters both ROS production and tumourigenicity [37]. However, recent large-scale sequencing efforts have failed to detect a relationship between specific mtDNA mutations and cancer, finding that mutations result from stochastic genetic drift as opposed to being selected for [38].

This study also demonstrated that the majority of mitochondrial mutations likely arise during mtDNA replication and are not because of oxidative stress [38].

*Mitochondria, "failed apoptosis" and cancer*

Besides ROS, mitochondria can impact on genome integrity through other means. As we have discussed, through the process of MOMP, mitochondria are required for apoptosis. The widespread nature of MOMP, such that it often occurs in all mitochondria, has led it being viewed as a cellular death sentence [4]. Recent work has challenged this idea, finding that MOMP can be engaged in minority of mitochondria, so-called minority MOMP [39]. Importantly, cells can survive minority MOMP but sustain caspase-dependent DNA-damage that can propagate genomic instability, transformation and tumourigenesis (Figure 2B). In line with this, loss of caspase-3 has been shown to inhibit tumourigenesis in a chemically induced cancer model of skin cancer [40]. These data argue that apoptosis also has a "dark-side" that can promote cancer through effects on genome instability.

Mitochondria and inflammation

The role of inflammation in cancer is complex - although inflammation can clearly have tumour inhibitory functions (for example by recruiting immune cells that target tumour cells) in many contexts inflammation has oncogenic effects; these include the supply of growth factors that promote proliferation as well as matrix-modifying effects that promote invasion and metastasis [41]. It is increasingly apparent that mitochondria play key roles in many aspects of inflammation, serving as sources on molecules that activate inflammatory pathways as well as signalling platforms to

propagate inflammatory signals. Here we review how mitochondria initiate inflammatory pathways through the release of various molecules before discussing how this may impact on cancer.

### *Mitochondria as a source of danger signals*

Our innate immune systems are geared to recognise diverse sources of danger, whether this be an invading bacterium or damaged tissue (which is often secondary to infection). Possibly stemming from their bacterial ancestry, mitochondria represent a source of several different molecules, termed damage associated molecular patterns or DAMPs, that serve to activate inflammation [42]. Mitochondrial DAMPs include mtDNA and N-formyl peptides. mtDNA, similar to bacterial DNA, contains hypomethylated CpG motifs that allow it to be recognised by Toll-like receptor, TLR-9. Consistent with a pro-inflammatory effect, direct injection of mitochondrial (but not nuclear) DNA induces neutrophil-dependent lung and liver inflammation [43] (Figure 3). N-formyl peptides bind the receptor FPR-1 to induce cytokine and chemokine production [44-46]. These DAMPs act in an extracellular manner but equally mitochondrial DAMPs can also act to engage intracellular signalling pathways. As discussed extensively, cytochrome c represents a potent mitochondrial DAMP that engages apoptosis, via binding to the adaptor molecule APAF-1, following mitochondrial release. mtDNA release from mitochondria can also elicit activation of various innate immune signaling mechanisms. For example, mtDNA can bind the NLRP3 inflammasome leading to caspase-1 dependent processing and secretion of the cytokines IL-1 $\beta$  and IL-18 [47] (Figure 3). How mtDNA can escape the mitochondria, leading to NLRP3 activation remains controversial. Some studies have

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argued that it occurs due to apoptotic mitochondrial permeabilisation but this has been challenged by others [47, 48]. mtDNA can also serve as an intracellular DAMP to activate the interferon pathway in a manner dependent on the signaling molecules cGAS and STING (Figure 3). Recent work demonstrates that mtDNA is released from mitochondrial under different stresses leading to STING-dependent interferon production [49-51].

Emerging evidence demonstrates a role for inflammatory mediators such as STING in the pathogenesis of cancer. For example, STING deficient mice are highly resistant to chemically induced skin cancer, in a manner that correlates with reduced inflammatory cytokine production [52]. Paradoxically, the loss of STING promotes colitis-associated cancer, possibly by enabling microbes to colonise damaged gut thereby triggering chronic inflammation [53]. While these, and other studies, demonstrate roles for inflammatory mediators in regulating cancer, an outstanding question is how important are mitochondria (through the release of DAMPs such as mtDNA) in these effects. This is challenging to address given that other DAMPs such as extranuclear, genomic DNA can also activate these pathways. Further understanding of how DAMPs such as mtDNA are released from stressed mitochondria leading to its inhibition, should enable this question to be addressed.

**Mitochondrial Roles in Invasion and Metastasis**

Cancer deaths are most often due to secondary tumours that arise by metastatic spread of cancer cells from the primary tumour. Metastasis necessitates various requirements of a tumour cell that include epithelial to mesenchymal transition,



stromal remodelling as well as invasion and migration of cancer cells. Focusing on two aspects of mitochondrial biology - bioenergetics and mitochondrial dynamics - we will now discuss how mitochondria impact on the metastatic process.

#### *Mitochondrial ROS as a metastatic driver*

The transcription factor HIF-1 $\alpha$  can be stabilized by mitochondrial ROS. In metastasis this may be important since HIF-1 $\alpha$  activity can lead to stromal remodelling and connective tissue degradation through matrix metalloproteinase (MMP) up-regulation, thereby providing space for cancer cells to migrate and invade into surrounding tissue [54, 55] (Figure 4A). Moreover, HIF-1 $\alpha$  can up-regulate VEGF vascular endothelial growth factor (VEGF) in doing so increasing tumour vasculature and facilitating entrance of tumour cells into the bloodstream [54, 56]. In support of a pro-metastatic role for mitochondrial ROS, it has previously been shown that mtDNA mutations in complex I can increase ROS production leading to angiogenesis and metastasis [33]. Furthermore, other work has shown that mitochondrial and oxidative phosphorylation, through the production of ROS, are essential for preserving the metastatic phenotype by regulating cell plasticity. Porporato and colleagues demonstrated that metastatic melanoma produce superoxide due to increased OXPHOS leading to electron transport chain overload. Mitochondrial ROS drive the activation of the tyrosine kinases Src and Pyk2, essential factors for the migratory plasticity of these cells [57] (Figure 4A).

#### *Mitochondrial dynamics and metastasis*

Mitochondria are highly dynamic organelles that undergo constant rounds of fusion and fission. Mitochondrial dynamics play many roles, not least in keeping the mitochondrial population healthy by complementing defective mitochondria or by enabling their removal through autophagy. Recent evidence also suggests that mitochondrial dynamics also play a crucial role in metastasis. Increased expression of the mitochondrial fission protein, Drp-1, has been observed in invasive and metastatic breast cancer [58]. Supporting a functional importance, inhibition of Drp-1 expression (leading to fused mitochondria) was found to inhibit invasion and migration. Interestingly, mitochondrial fission is required for formation of lamellopodia (the leading edge of migrating cells) [58] (Figure 4B). Because mitochondria accumulate in lamellopodia this suggests a localised enrichment of mitochondria is required for lamellopodia formation. Supporting these findings, another study has shown that mitochondrial distribution is asymmetric in cells migrating towards a chemotactic cue [59]. Specifically, mitochondria accumulate towards the leading edge of the migrating cell in a manner dependent on mitochondrial fission, since it can be inhibited by suppression of Drp-1 (Figure 4B). Importantly, in addition to regulating migration, mitochondrial fission has also recently been shown to be required for oncogenic transformation, suggesting that targeting fission components such as DRP-1 may have multiple anti-tumourigenic effects [60, 61].

Summary

In this review we have highlighted some of the ways in which mitochondria impact on tumourigenesis. Nevertheless, our discussion represents the tip-of-iceberg, with mitochondria playing multifaceted roles in almost every aspect of cancer biology.

Most importantly, our basic understanding of how mitochondria impact on cancer is already providing novel therapeutic approaches - for example the development of drugs that sensitise to mitochondrial apoptosis. Given the selective dependence of cancer cells on various mitochondrial roles, mitochondrial-targeting drugs should exert both potent and selective anti-cancer activity.

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### Figure Legends

**Figure 1. Mitochondria, cell death and cancer.** Following an apoptotic stress, BH3-only proteins are activated leading to BAX and BAK activation and mitochondrial outer membrane permeabilisation (MOMP). MOMP allows release of cytochrome *c* from the mitochondrial intermembrane space. In the cytosol, cytochrome *c* binds APAF-1 leading to its oligomerisation, caspase activation and apoptotic cell death. Cancer cells block apoptosis in various ways; loss of p53 can reduce their ability to

respond to specific apoptotic stresses or overexpression of anti-apoptotic BCL-2 proteins can prevent MOMP and cell death. Novel therapeutics, called BH3-mimetics, have been developed to neutralise anti-apoptotic BCL-2 function, thereby restoring apoptotic sensitivity to cancer cells.

**Figure 2. Mitochondria and genomic integrity.** A) Hypoxia or mutations in nuclear or mtDNA encoded mitochondrial proteins can lead to impairment of the electron transport chain and production of reactive oxygen species (ROS). ROS can lead to DNA mutations and deletions resulting in genomic instability. B) Sub-lethal stresses triggering minority MOMP can result in sub-lethal caspase activation promoting CAD activation and DNA damage, thus enhancing genomic instability.

**Figure 3. Mitochondria and inflammation.** mtDNA can be a robust inducer of inflammatory response in either intrinsic or extrinsic manner. Specifically, mtDNA can be released by dead cells and activate innate immune cells (neutrophils) acting as a DAMP (damage associated molecular pattern). Additionally, cytoplasmic mtDNA can activate both the NLRP3 inflammasome and the STING/interferon pathway leading to the production of various pro-inflammatory cytokines including IL1  $\beta$  and IFN $\alpha/\beta$ .

**Figure 4. Mitochondria in migration and metastasis.** A) Mitochondrial ROS can initiate the activation of the Src/Pyk2 kinases resulting in cytoskeleton remodelling and migration. Additionally, ROS can promote angiogenesis and matrix remodelling through the activation of HIF-1 targets, such as VEGF and metalloproteinases (MMPs). B) Mitochondrial fission in the leading edge of cells can drive migration and metastasis in a Drp1-dependent manner.

**Apoptotic stress**

**Loss of p53**

**Anti-apoptotic  
Bcl-2 proteins**

**BH3 mimetics**

**BAX**

**BAK**

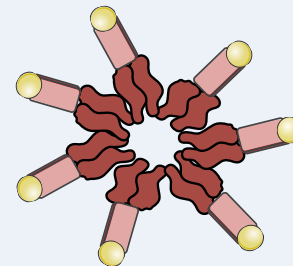
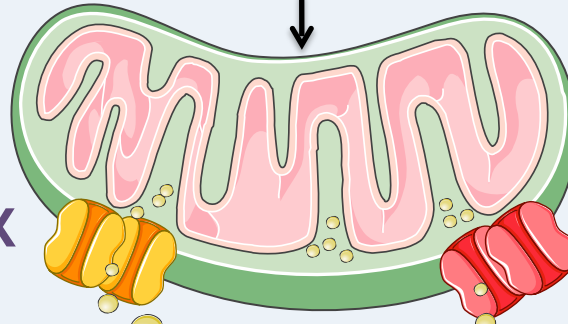
Mitochondrial  
Outer Membrane  
Permeabilisation  
(MOMP)

Cytochrome c

APAF-1 oligomerization

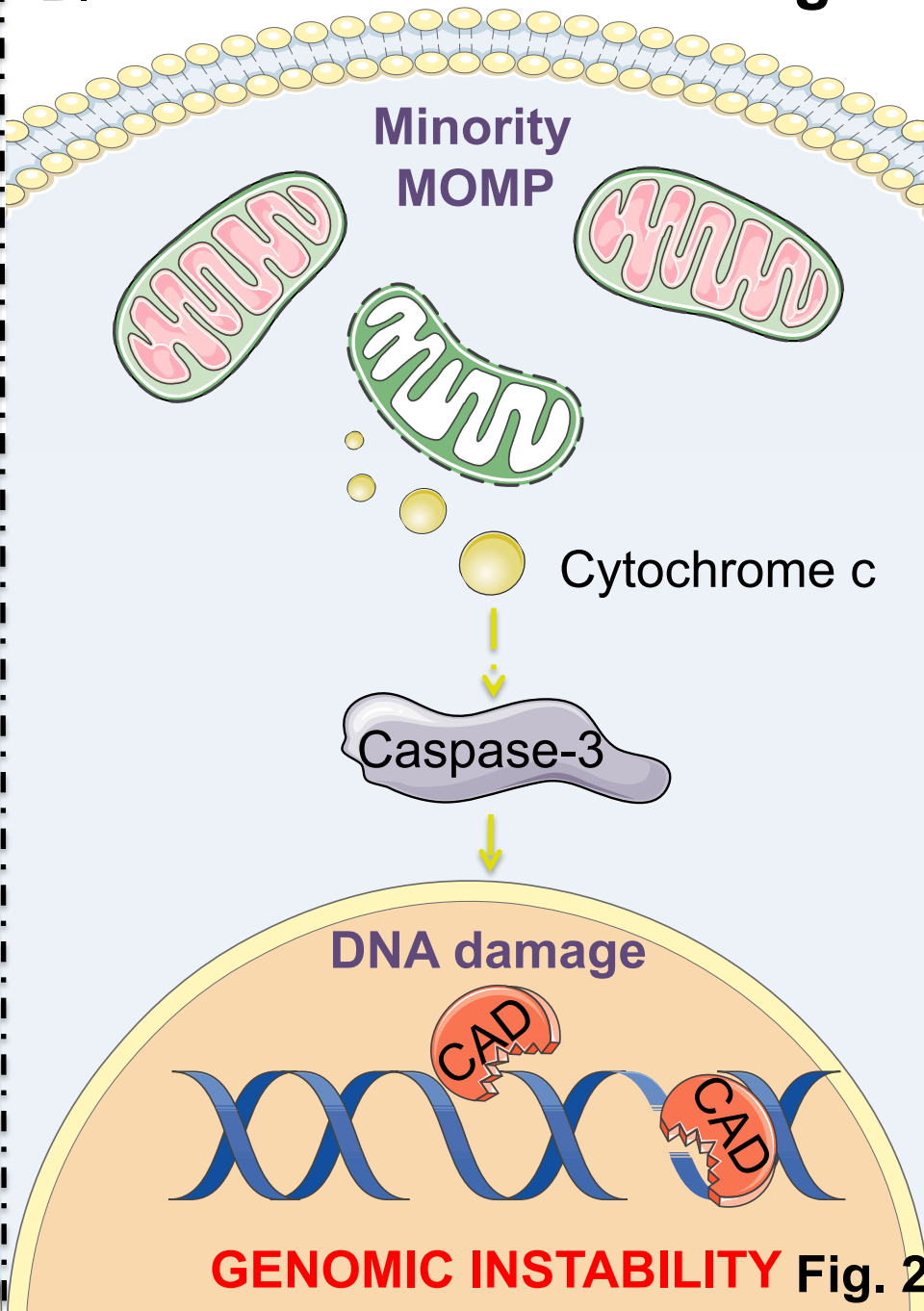
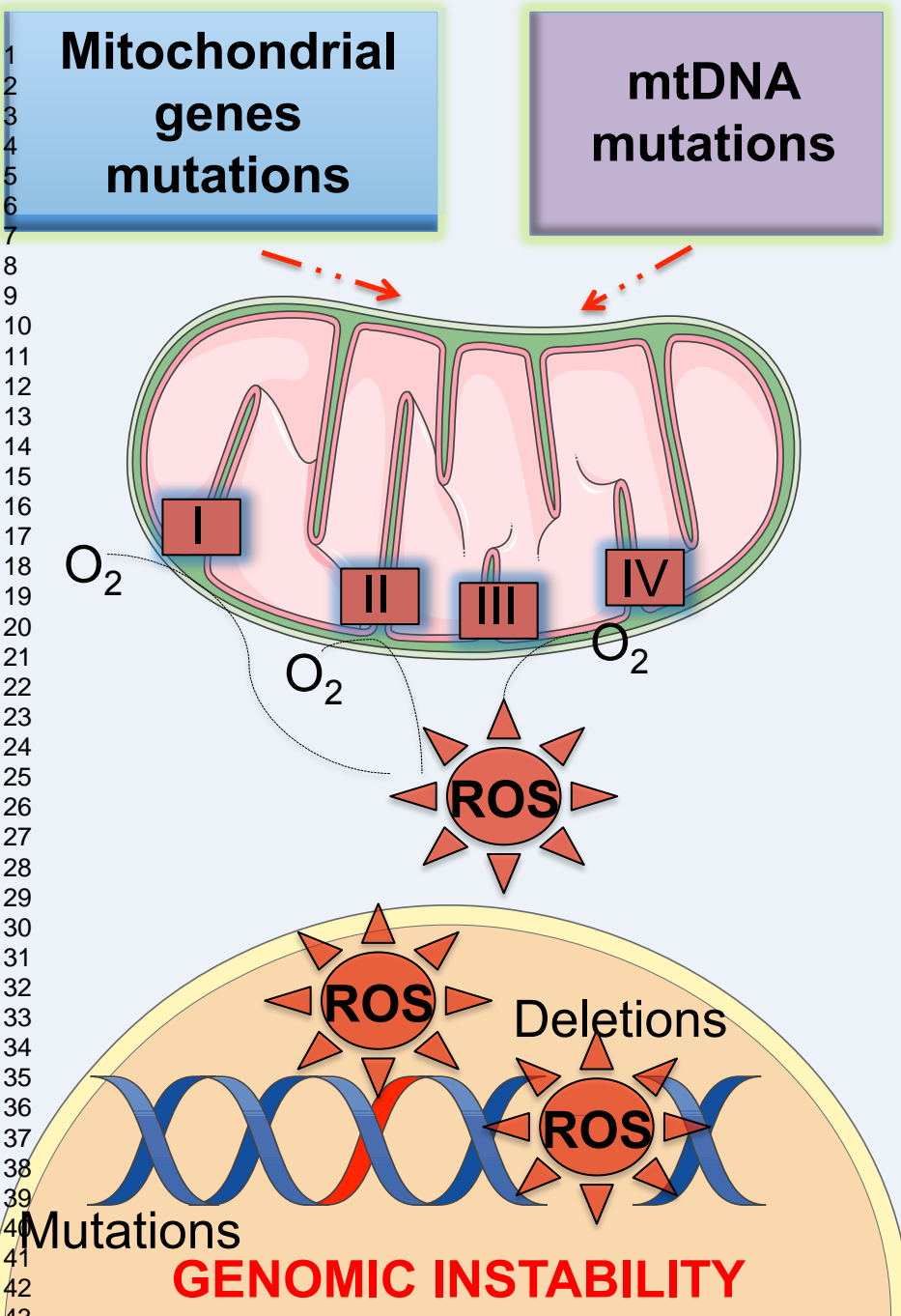
**Caspase activation**

**CELL DEATH**



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Fig. 2



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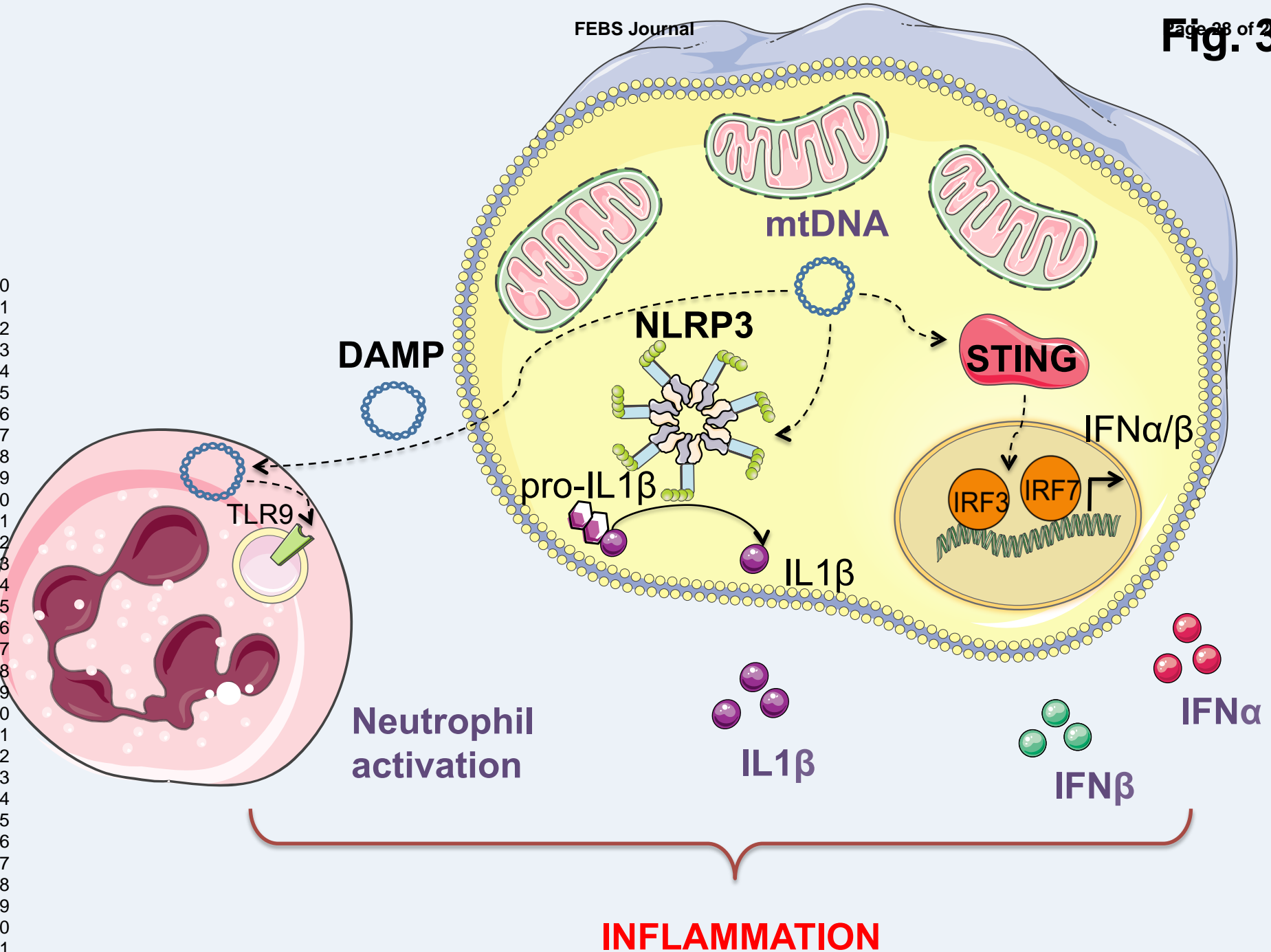


Fig. 4

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